

Title: Influence of N availability on interactions between Ascomycete fungi and Actinomycete bacteria during litter decomposition

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Project Goals: Atmospheric N deposition has increased N availability in natural ecosystems by 2-fold, on average, but as much as 100-fold in specific locations. Increased N availability has been widely reported to reduce decomposition of plant litter, at least on short time-scales. Studies of ecosystem response to N deposition have reported declines in plant litter decomposition rates and declines in the relative abundance of Basidiomycota fungi. Members of this phylum are root-associated plant symbionts, plant pathogens and plant biomass decomposers with key roles in acquiring N for plant growth and cycling N in surface soils. When Basidiomycota relative abundance declines, the relative abundance of Ascomycota fungi often increases. Thus, other fungi and bacteria can become more important in plant litter decomposition. Our work aims to determine how N gradients influence the activity and interactions of two broad decomposer groups---the Ascomycete fungi and Actinobacteria---that are known to harbor lignocellulosic decomposition traits and are potential key players in litter decomposition. While increases in relative abundance of these two groups have been documented in some studies, other studies suggest that increased N availability might inhibit Ascomycete activity.

We are monitoring the collective activities of mixed communities of Ascomycota and Actinobacteria in time course experiments, where defined mixtures of five fungal and five bacterial genera, decompose plant litter (arid-land grasses or pine litter) in sand microcosms under five nitrogen treatments. Given the difficulty of manipulating natural communities, defined mixtures provide the best approach to decipher functional responses, interactions, inherent biological barriers, and relevant mechanistic phenomena. To identify *general* patterns, instead of the eccentric response of one or two specific mixtures, we are documenting trends across many independent mixtures. Fungal and bacterial isolates for the mixtures were obtained from arid grassland sites and a pine forest field experiment where N application was an experimental variable. Measurements of the defined mixtures include initial biomass, CO₂ evolution over a 30-50 day time-course, initial and final community composition (rDNA surveys of fungal and bacterial composition), and metatranscriptome analyses.

Preliminary results show evidence of composition-dependent behavior. Nonetheless, strong patterns are emerging across fungal-bacterial mixtures that show a) the N concentration

can change the interaction between fungi and Actinobacteria and b) the phenomenon depends on the plant litter.

Cultures of five fungal of the mixed community species (*Aspergillus* CK392, *Chaetomium* CK152, *Coniochaeta* CK134, *Embellista* CK46, and *Phoma* CK108) were grown in the presence of different carbon sources: chitin, grass, sucrose and wood. Genomes were sequenced, assembled and annotated. Proteomic analyses of secreted proteins present in the culture supernatants were performed at EMSL, resulting in protein abundance data for each species and each carbon source. Initial analysis of the protein abundance data for the five fungal genomes revealed patterns of secreted protein abundance that correlated with the carbon growth substrate.

Comparisons of the most abundant proteins in the supernatants *Aspergillus* CK392 and *Coniochaeta* CK134 cultures revealed more total proteins present in the supernatants of the *Aspergillus* cultures than in *Coniochaeta* cultures. Examination of subsets of proteins in the *Aspergillus* cultures showed differences in the abundances of secreted enzymes, dependent on the carbon source. For example, the grass cultures showed higher levels of enzymes mediating degradation of complex polysaccharides, including cellulose, starch and xylan, which are all present in grasses.

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